

# Evaluation of the counter-immunoelectrophoresis technique in syphilis serology

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In the Netherlands, the majority of serological tests for syphilis are performed by the Regional Public Health Laboratories. The initial screening tests consist of a flocculation test (VDRL) and a complement-fixation test (Kolmer), both performed qualitatively. If one or both of these tests give a positive or a doubtful result, the Reiter protein complement-fixation (RPCF) test is performed. Sera still considered to present problems are then referred to the Treponemal Department of the National Institute of Public Health. As the two initial screening tests differ in technique but not in the nature of the antigen, one might argue for substituting one of them by a test using a treponemal antigen (Bissett, Browne, Coffey, and Michelbacher, 1961; Fiumara, 1963; Sequeira, 1959). Wilkinson, Scrimgeour, and Rodin (1972) reported favourable results with a combination of the automated reagin (AR) test and the RPCF test for screening purposes.

As most of the delay in reporting results is due to the complement-fixation test, a better substitute might be a test with a less tedious and time-consuming technique. We investigated as such the counter-immunoelectrophoresis technique (Gocke and Howe, 1970) using the Reiter protein antigen (RP-CIE test).

Some modifications of this test are described and the results compared with those of the quantitative RPCF test.

## Material and methods

All sera were provided by the National Institute of Public Health with the exception of those mentioned in Table I. The VDRL and Kolmer tests were performed according to the procedures recommended in the 'Manual of Tests for Syphilis' (1969). The RPCF test was done using 1·5 unit of complement and starting with undiluted serum (Bekker, de Bruijn, and Miller, 1966).

For the RP-CIE test, 7 × 7 cm. slides (Kodak) were used covered with 6 ml. of an 0·85 per cent. agarose solution (BDH) in 0·06 M barbital buffer pH 8·2. The wells had a diameter of 5 mm. and were placed at a

TABLE I *Effect of freezing and thawing on the rate of false-positive results in the RP-CIE test in 96 non-syphilitic sera*

Treatment of the sera	Number positive	Number negative
30 min. 56°C.	8	88
3 hrs -20°C.; 15 min. 56°C.	5	91
16 hrs -20°C.; 15 min. 56°C.	0	96

distance of 8 mm. (centre to centre) from each other. There was a left and a right row of six pairs of wells; the serum sample was put in the anodal well. The same buffer was used in both the anode and cathode vessel. A potential of 60 V was applied to the slide for 50 min., which gave about 4 mA per cm. Slides were washed for 15 min. in saline. The lysate of *Treponema reiteri* supplied by Dr. J. H. de Bruijn (Nat. Instit. of Publ. Hlth) was prepared by ultrasonic vibration of the washed sediment of the culture. It represents the CF antigen in the stage before freeze-drying (de Bruijn and Bekker, 1957) and was used in a concentration ten times stronger than that of the CF test.

All the serum samples subjected to the RP-CIE test had been inactivated at 56°C. for 30 min. They were frozen for at least 3 hrs at -20°C. and then gradually thawed, first at 4°C. and then at room temperature. The sera were subsequently further inactivated for 15 min. at 56°C.

The discounter-immunoelectrophoresis (DCIE) (Wallis and Melnick, 1971), which was initially used, was abandoned in favour of counter-immunoelectrophoresis; although the former had a greater sensitivity, it showed more non-specific precipitation lines.

## Results

### *Sensitivity, specificity, and reproducibility*

After its introduction (Bänffer, 1972) the test was modified to improve its sensitivity and specificity. The sensitivity could not be improved by varying the percentage of the agarose, the pH of the buffer, or the well diameter, or by using the sandwich technique (Smith, 1971). However, pre-electrophoresis of the serum sample for 5 min. before applying the antigen produced a precipitation line about halfway between the two wells and this was considered to be an improvement.

As regards specificity, in eight out of 96 fresh, non-syphilitic sera from healthy volunteer blood donors, non-specific precipitation lines were observed. This phenomenon could be avoided by the procedure of freezing and thawing the sera before inactivation described under Material and Methods (Table I).

The reproducibility of the RP-CIE test was tested by comparing the results of duplicate titrations of 63 sera done at different times (Fig. 1). 51 samples had the same titre in both assays. In four samples the titre was twice as high in the first assay and in eight samples the titre was twice as high in the second assay. There were no samples giving results differing by more than a factor of 2.

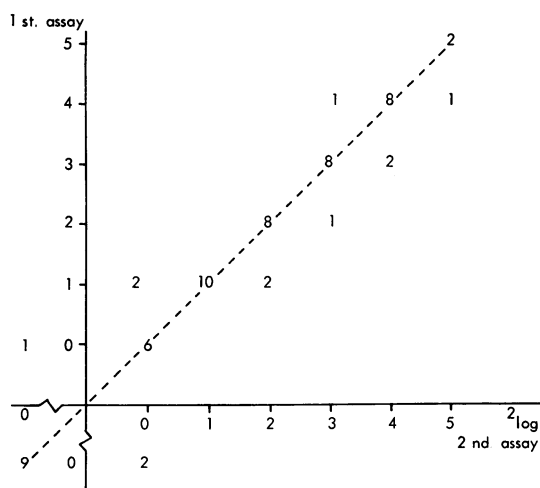


FIG. 1 Comparison of RP-CIE titres of 63 serum samples in assays performed at different times

#### *Correlation between the RP-CIE and RPCF tests*

The same antigen was used in the RP-CIE and RPCF tests. A comparison of the results of the qualitative tests has been published (Bänffer, 1972). Fig. 2 presents the geometric mean RP-CIE titres for different RPCF titre classes. The highest titre class was the only one in which less than eighty serum samples were titrated. From the data the correlation between the results in the quantitative tests is evident. RP-CIE titres are generally lower than RPCF titres.

We considered that problem sera might be better able to reveal differences between these two tests. Therefore we subjected 160 problem serum samples to both serological tests. The samples were selected because they were weakly reactive in some of the tests and negative in others. The results of this comparison are presented in Table II. The RPCF test was positive in eighty cases and the RP-CIE test

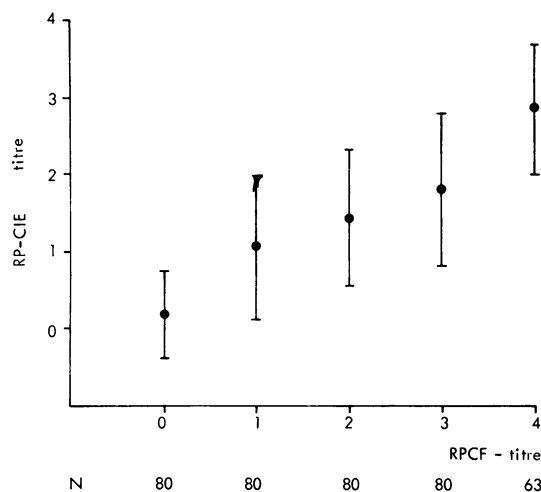


FIG. 2 Mean counter-immunoelectrophoresis titre ( $\pm$  S.D.) in Reiter protein complement-fixation titre classes ( $^2\log$ ).

N = number of cases

TABLE II Comparison of RP-CIE and RPCF test results in 160 problem sera

RPCF (1 : 1)	RP-CIE		Total
	+	—	
+	44	36	80
—	30	50	80
Total	74	86	

in 74. In 66 samples there was disagreement between the two tests.

Clinical information obtained in 58 of these cases is presented together with the serological results in Table III. The group of thirty serum samples positive only in the RP-CIE test included 23 cases of proven syphilis, whereas the group of 36 samples positive in the RPCF test included 21 cases of proven syphilis. In the same Table more information is given on the syphilitic cases. There were five cases of primary syphilis in the RP-CIE-positive/RPCF-negative group, against one in the RPCF-positive/RP-CIE-negative group of sera.

#### **Discussion**

Opinions on the usefulness of the RPCF test are contradictory. In two recent reviews it was given a rather poor rating (Sparling, 1971; Willcox, 1972) but this might have been because insufficiently sensitive techniques had been used (Bekker and others, 1966). According to the results of a survey of the British Cooperative Clinical Group (1972),

TABLE III Clinical information on sera showing discrepancy between RP-CIE and RPCF results

Diagnosis	RP-CIE- /RPCF+ (36 sera)		RP-CIE+ /RPCF- (30 sera)	
Syphilis	21		23	
Syphilis?	4		4	
Gonorrhoea	3		0	
No syphilis	2		1	
No information	6		2	

Stage of syphilis	RP-CIE- /RPCF+ Untreated		RP-CIE+ /RPCF- Treated	
Primary <sup>a</sup>	0	1	1	4
Secondary	1	4	1	1
Late	3	0	5	0
Latent	5	7	2	9

<sup>a</sup>All cases FTA-ABS positive

however, the RPCF test was performed routinely in 43 of the 68 participating laboratories in Great Britain. In the Netherlands the RPCF test has a definite place in syphilis serology (Bekker, 1960, 1962; Bekker and others, 1966).

From our results it appears that the specificity, sensitivity, and reproducibility of the CIE technique using Reiter protein antigen are comparable to those of the CF technique using the same antigen. The sensitivity of the CIE technique in the primary stage of syphilis seems to exceed that of the CF technique and may equal the sensitivity of the FTA-ABS test in these cases.

The RP-CIE test presents some advantages over the RPCF test, notably simplicity and speed of performance. In most cases a negative result can be reported in a few hours; positive samples are kept at

—20°C. and the assay is repeated after 16 hrs. The test is not hampered by the anticomplementary nature of the serum and does not exhibit a prozone phenomenon. The RPCF antigen is readily available commercially.

In conclusion, it may be said that the properties of the RP-CIE test justify its application as a screening procedure for syphilis.

## Summary

The counter-immunoelectrophoresis technique using Reiter protein as antigen has been evaluated as a serological test for syphilis. Some recent modifications are described. The test was compared with the quantitative Reiter protein complement-fixation test. It is concluded that the test is simple, relatively rapid, and reliable, and might therefore be used as a screening test for the serodiagnosis of syphilis.

## References

- BÄNFFER, J. R. J. (1972) *Lancet*, **1**, 996  
 BRITISH COOPERATIVE CLINICAL GROUP (1972) *Brit. J. vener. Dis.*, **48**, 254  
 BEKKER, J. H. (1960) *Ned. T. Geneesk.*, **104**, 1935  
 — (1962) *Brit. J. vener. Dis.*, **38**, 131  
 —, DE BRUIJN, J. H., and MILLER, J. N. (1966) *Ibid.*, **42**, 42  
 BISSETT, M. L., BROWNE, A. S., COFFEY, E., and MICHEL-BACHER, M. (1961) *Amer. J. publ. Hlth*, **51**, 1790  
 DE BRUIJN, J. H., and BEKKER, J. H. (1957) *Ned. T. Geneesk.*, **101**, 1615  
 FIUMARA, N. J. (1963) *J. Amer. med. Ass.*, **186**, 864  
 GOCKE, D. J., and HOWE, C. (1970) *J. Immunol.*, **104**, 1031  
 MANUAL OF TESTS FOR SYPHILIS (1969) Nat. Communicable Disease Center, Atlanta, Georgia  
 SEQUEIRA, P. J. L. (1959) *Brit. J. vener. Dis.*, **35**, 139  
 SMITH, J. B. (1971) *Int. J. Cancer*, **8**, 421  
 SPARLING, P. F. (1971) *New Engl. J. Med.*, **284**, 642  
 WALLIS, C., and MELNICK, J. L. (1971) *Appl. Microbiol.*, **21**, 867  
 WILKINSON, A. E., SCRIMGEOUR, G., and RODIN, P. (1972) *J. clin. Path.*, **25**, 437  
 WILLCOX, R. R. (1972) *Abstr. Hyg.*, **47**, 1073

## Immuno-électrophorèse comme réaction à antigène tréponémique

### SOMMAIRE ET CONCLUSION

En Hollande, deux réactions à antigène cardiolipidique—réactions de fixation du complément et de floculation—sont utilisées pour le dépistage de la syphilis. Au cas où une ou les deux réactions sont positives ou douteuses, la réaction de fixation du complément est effectuée avec l'antigène protéique de Reiter. Les réaction à antigène tréponémique n'interviennent donc pas en première ligne.

En appliquant l'immuno-électrophorèse et en utilisant l'antigène Reiter, une méthode rapide à antigène tréponémique a été établie. La précipitation spécifique se produit environ en une heure. L'introduction de l'antigène après électrophorèse préalable du sérum pendant 5 minutes augmente la netteté de la ligne de précipitation. La congélation du sérum à —20°C pendant 16 heures, suivie de décomplémentation par chauffage pendant 15 minutes à 56°C, élimine les précipitations aspécifiques.

Bien que les titres soient un peu plus bas, les résultats de l'immuno-électrophorèse concordent bien avec ceux de la fixation du complément à l'antigène Reiter.

D'une analyse de 160 sérums posant des problèmes diagnostiques, les conclusions suivantes ont été tirées :

- (1) La spécificité, la sensibilité et la reproductibilité des deux réactions sont comparables ;
- (2) L'immuno-électrophorèse a peut-être plus de valeur pour le dépistage de la syphilis primaire que la réaction de fixation du complément.

L'ensemble des résultats montre que l'immuno-électrophorèse peut servir comme réaction à antigène tréponémique de première ligne.